

9:00

### 831-3 $\beta$ -Adrenergic Stimulation Induces Cardiocyte Apoptosis Which Is Not Mediated by an Increase in Heart Rate

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**Background:** Both persistent tachycardia and  $\beta$ -adrenergic stimulation cause progressive heart failure. To explore possible mechanisms, we asked whether either stimulus was associated by apoptotic myocardial cell loss.

**Methods:** Four groups of rats treated for 24 hrs were compared: Control (C); Isoproterenol (Iso) of either 40  $\mu$ g/kg/hr or 400  $\mu$ g/kg/hr via Alzet minipump; mechanical pacing (500 bpm). Following treatment, hearts were formalin fixed, embedded in paraffin and cardiocyte apoptosis (Apo) was evaluated using TUNEL method on 5  $\mu$ m sections from the mid-ventricle wall.

#### Results:

	C	Iso 400	Iso 40	Pacing
HR (bpm)	424 $\pm$ 16	517 $\pm$ 21*	497 $\pm$ 8*	509 $\pm$ 6*
SBP (mmHg)	85 $\pm$ 6	82 $\pm$ 2	84 $\pm$ 7	97 $\pm$ 6
Apo (per area)	0.3 $\pm$ 0.3	7.9 $\pm$ 2.5*	0.8 $\pm$ 0.5	1.7 $\pm$ 0.7
Apo (per cells)	0.04 $\pm$ 0.04	1.26 $\pm$ 0.39*	0.13 $\pm$ 0.06	0.23 $\pm$ 0.08
Number of rats	5	4	6	5

(Data are mean  $\pm$  SEM. SBP, systolic blood pressure, per area, per cm<sup>2</sup>, per cells, per 10,000 cardiocytes. \*p < 0.05 vs. C.)

HR was increased by Iso 400, Iso 40, and pacing. SBP was similar amongst all four groups. Apo was increased by Iso 400, but not any the other treatments.

**Conclusions:** Thus, 24 hours of continuous  $\beta$ -adrenergic stimulation induces significant cardiocyte apoptosis and this effect is not mediated solely by an increase in HR.

9:15

### 831-4 Alterations in Myocardial $\beta$ -Adrenoceptor Subtypes and Signal Transduction Pathway in Nerve Growth Factor Overexpressing Transgenic Mice

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**Background:** We have established a transgenic mouse model in which selective overexpression of nerve growth factor (NGF) results in cardioselective sympathetic hyperinnervation and cardiac hypertrophy.

**Methods:** To determine whether the cardioselective sympathetic hyperinnervation alters the norepinephrine (NE) uptake function and myocardial  $\beta$ -adrenoceptor ( $\beta$ AR)-coupled adenylyl cyclase signal transduction system, we measured cardiac NE uptake site density (fmol/mg) by [<sup>3</sup>H]-nisoxetine binding, total  $\beta$ AR and  $\beta_1$ ,  $\beta_2$  subtype density (fmol/mg) by [<sup>125</sup>I]-iodocyanopindolol radioligand assay, dissociation constant ( $K_i$ , nM) of the  $\beta$ AR for isoproterenol, and maximum increase in cyclic AMP (cAMP, pmol/mg/min) produced by isoproterenol in 4 week-old control and transgenic mice.

#### Results:

	NE-uptake	$\beta$ AR	$\beta_2$	$K_i$	cAMP
Control	186 $\pm$ 55	35 $\pm$ 5	12 $\pm$ 2	0.17 $\pm$ 0.05	4.6 $\pm$ 0.8
NGF	1706 $\pm$ 221*	55 $\pm$ 5*	28 $\pm$ 3*	0.39 $\pm$ 0.07*	1.6 $\pm$ 0.2*

Values are means  $\pm$  SE. \*p < 0.05, vs. Control. N = 11.

**Conclusion:** Cardioselective sympathetic hyperinnervation in the transgenic mouse causes an increase in NE-uptake sites, an increase in  $\beta_2$ AR density, and no change in  $\beta_1$ AR density. The adenylyl cyclase response to isoproterenol, however, is reduced, probably because of the marked uncoupling of  $\beta$ AR to G-protein. The findings show that increased cardiac NE causes  $\beta$ AR subsensitivity without receptor downregulation.

9:30

### 831-5 Age-dependent Decrease in Human Cardiac $\beta$ -Adrenergic and Muscarinic Receptor Function: In Vitro and in Vivo Study

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In ageing cardiac  $\beta$ -adrenergic receptor (AR) function declines while little is known on muscarinic M<sub>2</sub>-R changes. To study possible mechanisms of  $\beta$ -AR and M<sub>2</sub>-R changes with age we assessed a) in vitro in right atria (RA) from 91 patients of different age (5 d-83 yrs) without apparent heart failure  $\beta$ -AR and M<sub>2</sub>-R densities. 10  $\mu$ M GTP, 100  $\mu$ M isoprenaline (ISO), 10 mM NaF

and 10 mM Mn<sup>2+</sup>-activated and carbachol (CAR 10 nM-100  $\mu$ M)-inhibited adenylyl cyclase (AC) activity and b) in vivo in 6 young (< 30 yrs) and 6 older (60 yrs) volunteers ISO-infusion (3.5-35 ng/kg/min)-caused increase and pirenzepine (PIR 0.32 and 0.64  $\mu$ g i.v. bolus)-caused decrease in heart rate (HR).

**Results:** 1)  $\beta$ -AR: In vitro  $\beta$ -AR density and -subtype distribution did not change while GTP, ISO, NaF and Mn<sup>2+</sup>-activated AC declined with age. In vivo ISO-infusion evoked HR-increases were not different between young and older volunteers; after pretreatment with atropine, however, ISO-evoked HR-increases were markedly enhanced in young but not in older volunteers. 2) M<sub>2</sub>-R: In vitro M<sub>2</sub>-R density was significantly negative correlated with age; concomitantly CAR-evoked AC-inhibition declined with age. In vivo PIR-induced decrease in ISO-evoked HR-increase was significantly reduced in the elderly.

**Conclusion:** In human RA  $\beta$ -AR and M<sub>2</sub>-R function decreases with age. The  $\beta$ -AR decrease is due to a reduced activity of AC-catalytic unit; the M<sub>2</sub>-R decrease is due to a reduced R-density. The decrease in M<sub>2</sub>-R density is accompanied by a blunted negative chronotropic response to M<sub>2</sub>-R stimulation in vivo, while the decrease in  $\beta$ -AR function can be demonstrated in vivo only when the counterregulatory action of parasympathetic activity is removed.

9:45

### 831-6 Echocardiographic and Histopathological Characterization of Young and Old Transgenic Mice Over-expressing the Human $\beta$ 1-Adrenergic Receptor

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**Background:** The  $\beta$ 1-adrenergic receptor ( $\beta$ 1-AR) is central to regulation of myocardial contractility. In the failing heart, the  $\beta$ 1-AR is chronically over-stimulated due to high concentrations of norepinephrine. Therefore, we wished to examine the effect of constitutive  $\beta$ 1-AR over-expression in myocardial tissues.

**Methods:** The human  $\beta$ 1-AR was overexpressed in transgenic (TG) mice, in a cardiac-specific context, using an  $\alpha$ -myosin heavy chain promoter. Receptor expression ranged between 1 and 2.5 pmol/mg membrane protein. Cardiac function (echocardiography) and histopathology (Trichrome and H&E) were examined at 4-5 months (young (yng)), and 8-20 months of age (old).

**Results:** Old TG mice had significantly greater left ventricular chamber sizes in both systole (LVDS) and diastole (LVDD) and a significantly reduced fractional shortening (FS) compared to young and old control and to young TG mice.

	CON-yng	TG-yng	CON-old	TG-old
FS (%)	58 $\pm$ 1	59 $\pm$ 1	60 $\pm$ 2	30 $\pm$ 4*
LVDS (mm)	1.5 $\pm$ 0.02	1.3 $\pm$ 0.1	1.3 $\pm$ 0.03	3.2 $\pm$ 0.4*
LVDD (mm)	3.5 $\pm$ 0.03	3.2 $\pm$ 0.1	3.4 $\pm$ 0.2	4.5 $\pm$ 0.3*

(\*p < 0.05 by ANOVA)

Histopathologically, both young and old TG mice had substantial myocardial remodeling with myocyte hypertrophy and large areas of interstitial replacement fibrosis.

**Conclusions:** Constitutive cardiac-directed over-expression of the  $\beta$ 1-AR in TG mice appears to produce a time-dependent remodeling resulting in ventricular dilatation and eventual cardiac failure. The  $\beta$ 1-AR transgenic mouse should be a highly useful model of adrenergically mediated dilated cardiomyopathy.

### 832 Coronary Blood Flow and Endothelial Dysfunction

Tuesday, March 31, 1998, 8:30 a.m.-10:00 a.m.  
Georgia World Congress Center, Room 254W

8:30

### 832-1 Effect of the Menstrual Cycle on Endothelium-dependent Vasodilation of the Brachial Artery

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**Background:** Estrogen alone or in concert with progesterone therapy has beneficial effects on endothelium-dependent vasodilation in postmenopausal women and such improvement in endothelial function may explain the reduction in cardiovascular events associated with hormone replacement therapy.